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COLLAGEN

Collagen is the principal structural protein in the vertebrate body. The extracellular proteins of the main connective tissues consist of 90% or more collagen in tendon and bone, and more than 50% in the skin. The different properties of these tissues are in part the result of different architectural organizations of collagen fibrils. This protein is absent from single-celled animals and from plants where cellulose and related polysaccharides serve a similar role. However, collagen is found throughout the invertebrates. For example, the once common natural bath sponge is largely collagen. In animals with a mineralized (*Mollusca*) or chitinous (*Arthropoda*) exoskeleton, collagen is found in association with internal organs. Collagen is also present in the body wall and cuticle of other invertebrates. It is no wonder that collagen, an important natural biological

material, has been extensively studied as a polymer for use in manufactured materials (see also PROTEINS).

Historically, technology of collagen in the form of leather and gelatin goes back millennia. Leather (qv) is, of course, chemically treated animal skin. Gelatin (qv) is heat and chemically denatured and degraded animal connective tissue. Both consist largely of collagen but are very different in chemistry and form (1). Commercial interest in these materials provided the stimulus for research on collagen prior to about 1950. Since that time collagen has been intensively studied because of its biological importance.

The term collagen usually implies the collagen present in skin, tendon, and bone. At present, ten different vertebrate collagens have been identified (2–4), and other types will undoubtedly be described in the near future. All collagens contain a unique triple helix; however, the length of the helix and the nature and size of nonhelical portions of the molecule vary from type to type. The predominant collagen of skin, tendon, and bone is type-I collagen; type-II collagen is essentially unique to cartilage; and type-III collagen occurs in adult skin (5–10%) in association with type I and may be a minor contaminant of type I collagen prepared from this source. The other types occur in small amounts and are usually associated with specific biological structures. Only type-I collagen is discussed here unless specifically noted, and for convenience is referred to without a type designation. Types II and III collagen are structurally very similar to type I, and much of what applies to type I applies to types II and III.

The term collagen is usually used generically and may apply to the molecule, the native fibril as it exists *in situ* or is reconstituted *in vitro*, one of several polymorphic aggregates, or simply to bulk material of unspecified structure. Although this terminology is a useful shorthand and the specific form of the collagen can often be inferred from the context, in many cases it is necessary to specify the form.

Collagen is, of course, a protein of a size and amino acid sequence determined by the genes of the organism and by biological processing steps that regulate its final chemistry. It has specific helical and aggregate structures, again biologically regulated. Thus collagen is very unlike most synthetic or natural polymers, which contain chains of variable length composed of one or two repeating units and whose physical and chemical properties are, to a considerable extent, determined by manufacturing steps. Of course, once isolated, the properties of collagen can also be altered, but an understanding of the native chemistry and structure is necessary. Significant alterations produce new materials, as already noted for leather and gelatin.

Chemistry

Chain Composition. The type-I collagen molecule contains three polypeptide chains, termed α chains; two are of one kind, termed $\alpha 1$(I), and one is of another, termed $\alpha 2$(I). They are similar and homologous. Both consist of repeating triplets of sequence glycine-X-Y where X and Y can be any amino acid; proline is frequently in the X position, and hydroxyproline in the Y position. Hydroxyproline is derived from proline posttranslationally and thus their positions are

a result of enzymatic specificity. The α1(I) chain has 1056 residues of which 1014 are continuous triplets. Nontriplet regions, termed telopeptides, consist of 16 residues at the N-terminal end and 26 residues at the C-terminal end. The α2(I) chain also has 1014 residues in continuous triplets, but the N-terminal nontriplet region contains 9 residues and the C-terminal nontriplet region contains 15 residues for a total length of 1038 residues.

The function served by the nonidentity of the three chains is not known. The closely related types II and III collagen have three identical chains (α1(II) and α1(III), respectively), and α1(I) forms a trimer under some conditions both *in vitro* and *in vivo*. However, the homotrimer is only partially equivalent functionally to the heterotrimer and is considered an aberrant form.

Amino Acid Composition. The amino acid composition of collagen is unusual in several respects. The compositions of the α1(I) and α2(I) chains of calf-skin collagen are given in Table 1. These values were assembled from the known amino acid sequence (2) and thus represent theoretical analytical values. Species differences among mammals are small. Quantitatively, one third of the residues are glycine and another 23 residue % (in mammals) are proline or hydroxyproline.

Table 1. Amino Acid Composition of the α Chains of Calf-skin Collagen (Type I)[a]

Amino acid	α1(I)[b]	α2(I)[b]
alanine	124 (2)	111 (3)
arginine	53 (2)	56 (1)
asparagine	13	23
aspartic acid	33 (3)	24 (2)
glutamic acid	52 (2)	46 (2)
glutamine	27 (3)	24 (1)
glycine	345 (6)	346 (6)
histidine	3 (1)	8
hydroxylysine	4	9
hydroxyproline[c]	114	99
isoleucine	9 (1)	18
leucine	22 (3)	33
lysine	34 (2)	21 (1)
methionine	7	4
phenylalanine	13 (1)	15 (3)
proline[c]	127 (4)	108 (1)
serine	37 (5)	35 (1)
threonine	17 (1)	20
tyrosine	5 (5)	4 (3)
valine	17 (1)	34
Total	*1056 (42)*	*1038 (24)*

[a] Data courtesy of Klaus Kühn, Max Planck Institute for Biochemistry, Munich, FRG.

[b] Residues per α chain. The values in parentheses are the residues contributed by the nonhelical ends. Values were calculated from the known sequence and represent theoretical values.

[c] Analytical values for hydroxyproline are lower and for proline higher than those given here because of incomplete hydroxylation (90–95% average) at hydroxyproline sites.

These amino acids are critical to the molecular structure. Qualitatively, hydroxyproline and hydroxylysine are essentially unique to collagen. Hydroxyproline is always present in mammalian collagen at about 9–10 residue %. Hydroxylysine content varies from about 0.5 to 2 residue %, depending on species, tissue, and sometimes the age of a given tissue. Since hydroxylysine and its precursor lysine participate in covalent cross-links but have different chemistries, this variation may affect tissue properties. In other respects the amino acid composition is unremarkable. Tryptophan and cystine are absent from type-I vertebrate collagen but may be present in other collagens.

Carbohydrate Content. The carbohydrate content of type-I collagen is less than 1% in pure preparations. The chemical form is unique to collagen, consisting of β-D-galactopyranosylhydroxylysine and α-D-glucopyranosyl-(1→2)-β-D-galactopyranosylhydroxylysine (5). The linkage to hydroxylysine is between C-1 of the galactose and the δ-hydroxy group of hydroxylysine, as shown in Figure 1. The hydroxylysine residues are known to be residues 87 and 930 in the triplet region of the α1(I) chain but have not been identified in the α2(I) chain (5). The sites are not always glycosylated and may sometimes carry the monosaccharide and sometimes the disaccharide. The function of the carbohydrate is unknown. It may be significant that the same hydroxylysine residues in α1(I) are involved in covalent cross-links, but there is no evidence that the carbohydrate participates.

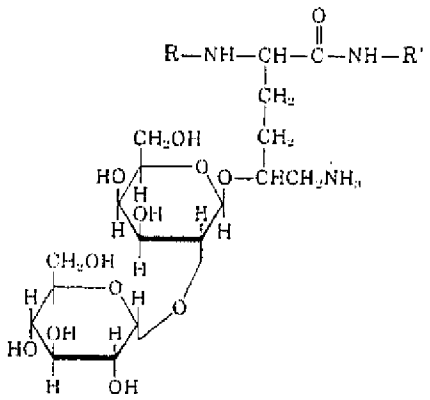
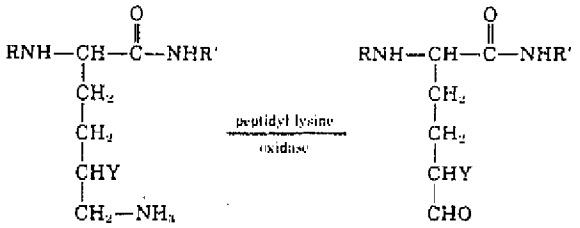


Fig. 1. Structure of the carbohydrate, peptidyl glucosylgalactosylhydroxylysine, specific to collagen; R and R′ represent popypeptide chains.

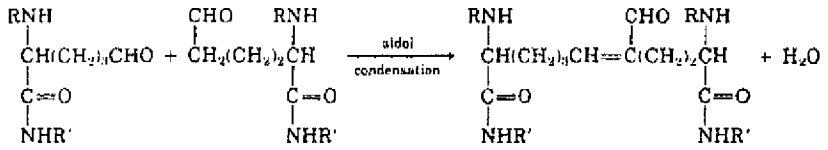
Cross-links. Under most conditions, collagen in tissues cannot be solubilized without degradation because the molecules are covalently cross-linked. The strength of collagenous tissues is markedly reduced if cross-linking is inhibited. The best evidence for this is provided by lathyrism, a disease induced in experimental animals by feeding a lathyrogen that inhibits the cross-linking enzyme peptidyl lysine oxidase (6). In this condition the partial blocking of cross-linking results in connective tissue that is easily disrupted by stress. The cross-links in collagen are unique to this class of proteins except for elastin (2,7):

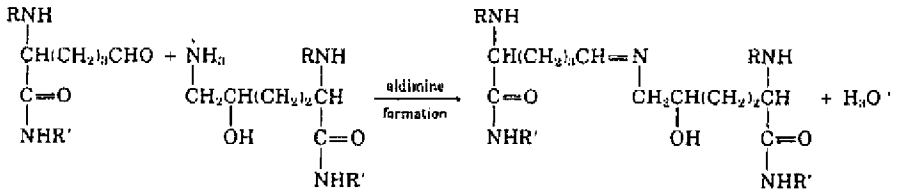
The first step in cross-linking is the enzymatic conversion of lysine or hydroxylysine residues in the telopeptide end regions of the collagen molecule to the aldehydes allysine or hydroxyallysine:



where R and R′ represent polypeptide chains, and Y may be H (lysine and allysine) or OH (hydroxylysine and hydroxyallysine).

This reaction is carried out by a specific enzyme, peptidyl lysine oxidase. Its substrate is an aggregated form of collagen; the conversion occurs during fibril formation. At that time, molecules in the fibril are associated in such a way that the reactive aldehydes allysine and hydroxyallysine can spontaneously condense (aldol condensation) or react with the ϵ-amino groups of nearby lysine or hydroxylysine residues (aldimine formation):





where R and R′ represent polypeptide chains. These interchain bifunctional cross-links, which may be intra- or intermolecular, are still reactive and continue to form polyfunctional cross-links through multiple condensations that are still incompletely characterized. Indeed, it is likely that in most tissues, cross-links bind all the molecules in the fibril together. Because the initiating reaction is with specific residues and molecules have a fixed staggered relationship, regions of cross-links are repeated at regular intervals along the fibril. As already noted, these residues are in the telopeptide ends and at positions 87 and 930 in the helical region of the α chains. Nearby histidine residues may also be involved.

Structure

Molecule. The triple-chain helix is a unique structure that defines a protein as a collagen. The stability of the helix depends on two factors: glycine (Gly) as every third amino acid in the amino acid sequence and proline and hydroxyproline as frequent occupants of the X and Y positions, respectively, of the repeating Gly-X-Y triplet. The absence of a side chain on glycine permits triple-

chain packing as these residues fall in the middle of the helix where there is no room for a side chain. Proline contributes to helical stability through its conformation-directing properties. (Polyproline in aqueous solution forms a single-chain left-handed helix similar to the helix of one chain in a collagen helix.) Hydroxyproline contributes to stability apparently through water-bridged hydrogen bonds, but these structures are not well-defined. This view of stability is somewhat simplified as other factors play a role (8,9).

A short segment of collagen helix is shown in Figure 2. The glycine residues, contributed alternately by the three α chains, occupy the middle of the structure. The side chains of the other residues are directed outward where they can interact with side chains on other molecules to determine molecular packing. The residue spacing in the collagen helix is 0.286 nm, which gives rise to a characteristic reflection on the high angle x-ray diffraction pattern. Supercoiling of the α chains results in prominent reflections on layer lines at 0.4 and 1.0 nm. These features

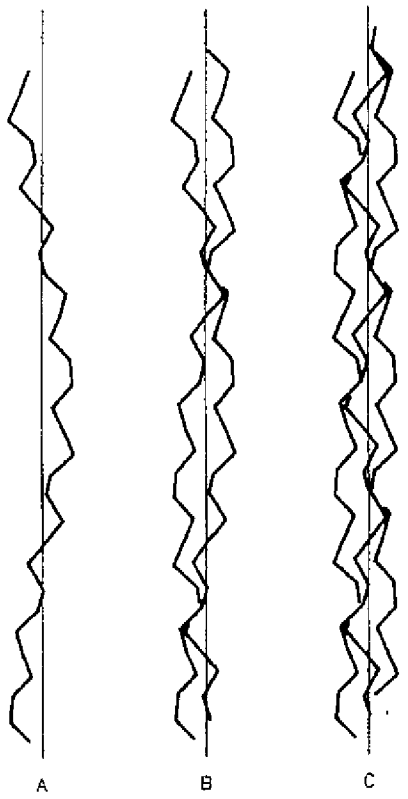


Fig. 2. A short section of collagen triple helix (30 amino acid residues) illustrated by showing: A, one chain; B, two chains; and C, three chains. All three chains are necessary for stability. Only the backbone carbon positions of the amino acids are shown. Amino acid side chains extend outward from the polypeptide backbones. Computer drawn image courtesy of B. L. Trus, Division of Computer Research and Technology, National Institutes of Health.

are characteristic of the triple helix. The three-residue helix of the individual chains is left-handed and the supercoil is right-handed. The pitch of the supercoil is not known but has been estimated to be about 100 nm (30–40 residues). Thus, the helical portion of the collagen molecule with 1014 residues in each of its three α chains is 290 nm long (1014 residues × 0.286 nm per residue). The short nonhelical ends add an additional few nanometers, depending on their conformation. This calculated value is very close to the value of about 300 nm measured by electron microscopy of individual molecules or of fibrils. The diameter of an equivalent solid cylinder can be calculated to be 1.1 nm, but side-chain extensions result in effective packing diameters of about 1.5 nm as measured by x-ray diffraction of rat-tail tendon.

Native Fibril. The D-periodic banded fibril seen by electron microscopy is one of the best-known characteristics of type-I collagen (9–11). Negatively stained fibrils from rat-tail tendon are shown in Figure 3. The pattern is polarized indicating that collagen molecules, which are approximately parallel to the fibril axis, all point in the same direction within a fibril. The D period measured on electron micrographs is about 64 nm. However, drying and fixing results in shrinkage. The more accurate value is 67 nm as measured by x-ray diffraction of oriented, wet fibrils in tendon stretched just enough to remove slack. Because the collagen molecule is about 300 nm long, it can be readily calculated that one

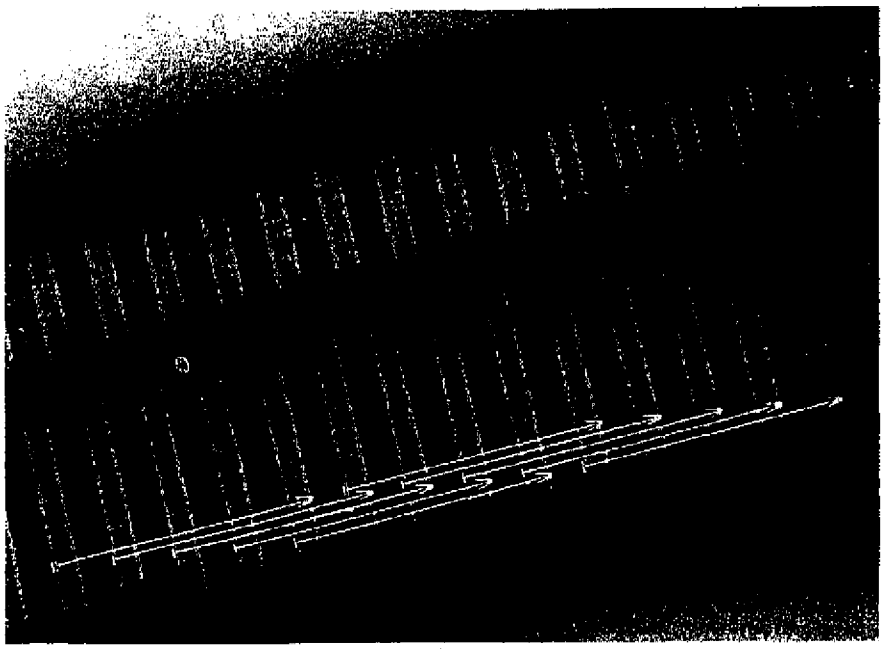


Fig. 3. Electron micrograph of fibrils from rat-tail tendon negatively stained with phosphotungstic acid; the periodicity is 67 nm. The superimposed arrows represent staggered collagen molecules 300 nm long showing their axial relationship in the fibril. The arrows point in the C-terminal direction; the two fibrils have opposite polarities (12). Courtesy of the American Association for the Advancement of Science.

molecule passes through about 4.5 D periods (4.4 by electron microscopy; 4.47 by x-ray diffraction). The D period arises from a regular stagger between adjacent molecules of multiples of D (Fig. 3). Statistically then, a fibril contains gap and overlap regions since the ends of molecules in linear alignment do not meet. The overlap regions contain more protein than the gap regions, which results in greater retention of stain in the latter when negatively stained. The dark (0.4 D) and light (0.6 D) bands (Fig. 3) are thus readily explained.

Because of the following facts: the amino acid sequences of the α chains of type-I collagen are known; the D period can be defined as 234 amino acid residues (67 nm/(0.286 nm/residue)); and positive staining shows the positions of charged amino acid side chains, the position of every amino acid in the sequence can be assigned to a band (or interband) on electron micrographs of positively stained fibrils (Fig. 4). Indeed, if the known positions of charged residues in the sequence are plotted as lines on a linear scale and if that scale is superimposed on itself graphically with 234 $\times$ n (n = integer) staggers, the band pattern of the fibril

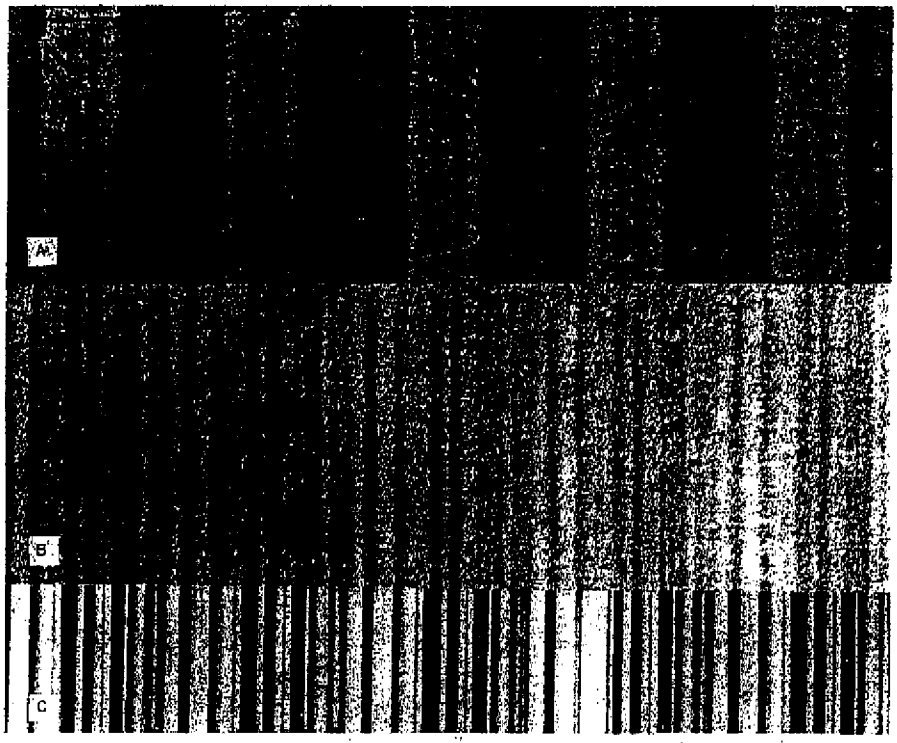


Fig. 4. A, Electron micrograph of a negatively stained collagen fibril with a superimposed arrow representing a collagen molecule, as in Figure 3. B, Electron micrograph of a positively stained collagen fibril. The two patterns can be aligned because of light positive staining superimposed on the negative stain (A). C, Band pattern reconstructed photographically from the sequence of charged amino acids (lysine, arginine, glutamic acid and aspartic acid) assuming molecules to be placed as shown by the arrow (A) and staggered as shown in Figure 3 (11). Courtesy of the *Journal of Supramolecular Structure*.

can be reproduced (11,13), as shown in Figure 4. The one-dimensional or projected structure of the fibril is thus understood to high resolution.

The lateral relationship between molecules in the native fibril is less certain. The x-ray diffraction pattern usually shows minimal equatorial information with the principal reflection at about 1.3 nm. For some tendons, however, under light stretch the 1.3-nm equatorial reflection can be shown to contain three maxima, and several row-line reflections at longer spacings appear. These data have been used to define a unit cell containing one molecule related by quasi-hexagonal packing to other molecules (14,15). Although the unit cell is now agreed upon, the arrangement of the contents is uncertain.

Other information gives some idea of how the contents may be organized. Electron microscopy of polymorphic forms of collagen aggregates strongly suggests that the native fibril has a regular substructure that is also D periodic and asymmetric. What these elements are is not known, but the simplest model is a fivefold microfibril (Fig. 5). In this model, fibrils would be bundles of microfibrils (16). In the general case, for which there is some evidence, molecules in the microfibril would be supercoiled, and microfibrils could also be supercoiled. The several levels of coiling create a ropelike structure.

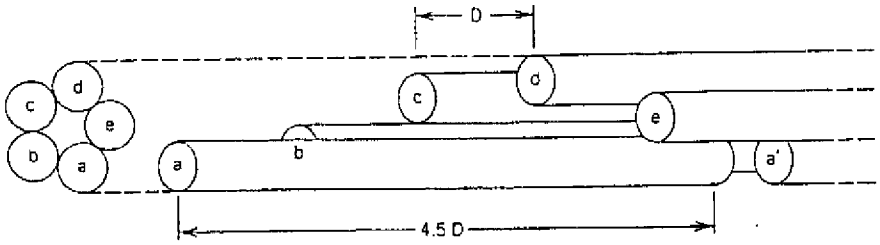


Fig. 5. A schematic representation of a fivefold microfibril (16). The rods represent collagen molecules 300 × 1.5 nm with the projected stagger relationship shown in Figure 3. The molecules are supercoiled in the actual structure. The handedness of the helix is not known. The model assumes that collagen fibrils are composed of bundles of parallel, aligned microfibrils.

Whatever the correct packing arrangement as defined by covalent structure, it is not normally crystalline in the lateral dimensions. Nuclear magnetic resonance measurements show that molecules in a fibril undergo rapid rotational reorientation about their axes (17). Model studies suggest that this motion is segmental through an angle of about 30° with a rotational diffusion coefficient of about $10^7\ s^{-1}$. Crystalline lateral order may arise from the compression of bundles of microfibrils, forcing segments of molecules onto the quasi-hexagonal lattice defined by x-ray diffraction (18).

Other Aggregates. Collagen can be precipitated in several polymorphic forms in addition to the native fibril. Although these forms have no known biological relevance, they have been useful in determining native fibrillar structure and understanding molecular properties (9,19,20). Polymorphic fibrillar forms include four different D-periodic symmetric fibrils, a set of D-periodic obliquely banded fibrils, and filament-long-spacing fibrils. The D-periodic symmetric fibrils

may consist of D-periodic asymmetric microfibrils in antiparallel array with four different stagger relationships. The obliquely banded fibrils appear to consist of parallel D-periodic subfibrils (narrow fibrils within a wide fibril) associated side to side with a 10-nm step. The subfibril width, and thus the angle of the band, is variable. The filament-long-spacing fibrils are composites of collagen molecules and a polyanion such as chondroitin sulfate.

Another aggregate useful in characterizing collagen is the segment-long-spacing (SLS) form (21). If collagen in acid solution is mixed with a multivalent anion such as adenosine triphosphate (ATP) or another triphosphate, the positively charged collagen rods associate with ends aligned through bridges formed by the anion. The SLS structure, much like a bundle of pencils, can be stained and viewed by electron microscopy (Fig. 6). The band pattern arises from charged amino acid side chains and thus reflects the primary structure.

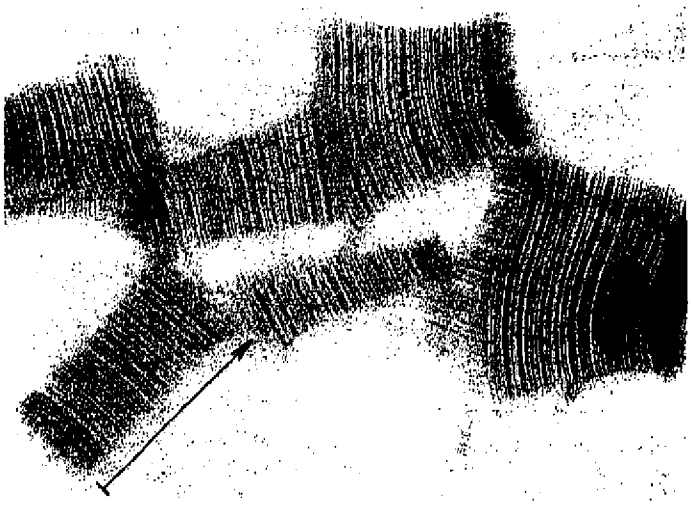


Fig. 6. An electron micrograph of positively stained segment-long-spacing (SLS) aggregates of collagen molecules. The SLS aggregates are bundles of parallel, aligned collagen molecules 300 nm long. The arrow represents a collagen molecule pointing in the C-terminal direction. The SLS band pattern arises from positively charged amino acids, and when molecules are staggered as in Figure 3, gives rise to the fibril band pattern shown in Figure 4B (9). Courtesy of Elsevier Science Publishing Co., Inc.

Tissue Architecture. The relation between morphology, molecular structure, and biological function of connective tissue is discussed in Ref. 22. Collagen used in the form of intact tissue has properties determined in part by tissue architecture, and thus the subject may be of importance to the chemist and engineer. The biomechanics of collagenous tissues is given in Refs. 23 and 24.

Biosynthesis

Type-I collagen is made as a precursor, type-I procollagen, which is longer at both the N- and C-terminal ends. A schematic representation of a procollagen

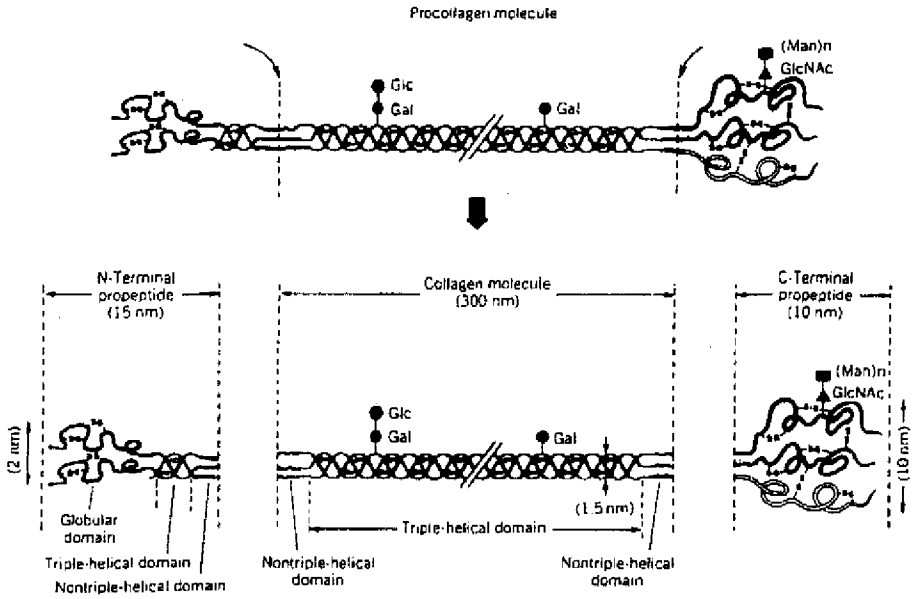


Fig. 7. Type-I procollagen molecule and the cleavage products from its conversion to type I-collagen. The N-terminal propeptide contains a short helical region and intrachain disulfide bonds. The C-terminal propeptide contains N-linked carbohydrate typical of glycoproteins and both intra- and interchain disulfide bonds. The collagen molecule has short nonhelical ends and collagen-specific carbohydrate (Fig. 1) (25). Gal = galactose; Glc = glucose; GlcNAc = N-acetylglucosamine; and Man = mannose. Courtesy of the *New England Journal of Medicine*.

molecule is given in Figure 7. The biosynthesis of type-I procollagen is similar to other proteins made to be secreted. It involves transcription of genes, processing and translation of mRNA, processing and folding of polypeptide chains, transport through the cell and secretion, extracellular processing, and assembly into the macromolecular form (26–28). Details of these steps are given below.

1. Events involving nucleic acid to yield prepro α chains:
   - Transcription of the α1(I) and α2(I) genes.
   - Splicing, capping, and polyadenylation of pre-mRNA.
   - Translation of mRNA.
2. Intracellular processing to yield procollagen:
   - Removal of prepeptide.
   - Hydroxylation of proline.
   - Hydroxylation of lysine.
   - Glycosylation of hydroxylysine.
   - N-linked glycosylation of propeptide.
   - Chain association and propeptide folding.
   - Disulfide bond formation.
   - Triple-helix formation.
   - Transport and secretion.
3. Extracellular processing to yield collagen fibrils:
   - Proteolytic conversion of procollagen to collagen.

Fibril formation.
Aldehyde formation.
Covalent cross-linking.

Transcription and Translation. Procollagen genes isolated from vertebrate species are unusual; only about 10% of the DNA sequence codes for a pro α chain. The remaining 90% must be spliced from the pre-mRNA after transcription. The coding regions are commonly 54 base pairs in length, corresponding to 18 amino acids and suggesting a primordial gene or fundamental unit of this size. However, the unit is unrelated to protein structure or function, insofar as is known.

Intracellular Processing. The prepeptide of procollagen, necessary for transmembrane synthesis of proteins destined for secretion, seems to be longer than those for most proteins but serves the same function. Hydroxylation of proline and lysine and glycosylation of hydroxylysine begin as nascent chains are formed by translation. These steps must all occur prior to helix formation because only random chains are substrates for the enzymes; once helix formation is complete, they stop. Because helix stability is dependent on hydroxyproline, helix formation and proline hydroxylation are interdependent and self-regulating. On the other hand, lysine hydroxylation and glycosylation, as already noted, can and do vary in extent. Glycosylation in the C-propeptide region results in a high-mannose, asparagine-linked oligosaccharide typical of most secreted proteins. Chain association begins in the C-propeptide region and is rapidly followed by disulfide bond formation in the same region and by triple helix formation. Transport and secretion occur in vacuoles in the same manner as other secreted proteins.

Extracellular Processing. These steps as they occur *in vivo* are poorly understood, although all have been studied *in vitro*. Procollagen is converted to collagen by two proteolytic enzymes, one specific for the C-propeptide and the other for the N-propeptide (see Fig. 7). Assembly into fibrils is spontaneous *in vitro* but must be regulated and coordinated with lysine and hydroxylysine oxidation *in vivo*. Once these steps have occurred, cross-linking is apparently spontaneous *in vitro* and *in vivo* but may take days or weeks to be fully complete. *In vitro* no other molecules, large or small, need be associated with collagen fibrils, but this has not been shown *in vivo*. Biological regulation may require the participation of other molecules, perhaps on cell surfaces.

Immunology

The immunology of collagen is important for two reasons (29,30). First, because animal collagens are being used in humans, it is necessary to understand the potential immunological consequences. Second, antibodies to collagen and other proteins are powerful tools used in the laboratory for highly specific and sensitive assays (see also ANTIBODIES AND ANTIGENS).

Immunogenicity. The term immunogenicity relates to the capacity of a material to elicit the production of antibodies in animals or humans. Generalizations concerning immunogenicity are of little use because the immune response depends not only on the nature of the material but on the individual animal as well as the species and on the frequency and route of administration. Thus, statements that collagen is nonimmunogenic or has low immunogenicity must

be made in the context of these variables. However, the impression gained by many investigators that collagen does not readily elicit antibodies compared to other common proteins is undoubtedly true. The reason for this behavior seems to be that species differences among type-I collagens are small. Unlike globular structures, the triple-helical conformation is not subject to even minor variation. Thus, all type-I collagens from mammalian species are very similar and are not readily viewed by the immune system as foreign. The short telopeptide ends of collagen may be an exception since they are probably unstructured and show considerable species variation. As discussed below, they do contain major antigenic determinants. Denatured collagen presents still another picture. It would be expected to be immunogenic because it is not normally present and would be viewed as foreign. On the other hand, denatured collagen is rapidly metabolized and cleared, and exposure would be brief.

Recent experience with an injectable fibrillar collagen prepared by reconstitution of pepsin-solubilized bovine-skin collagen is the only source of systematically collected information about collagen immunology in humans. This collagen, as a 35 or 65 mg/mL suspension of reconstituted fibrils (Zyderm Collagen Implant, Collagen Corporation), is injected intradermally to correct soft-tissue-contour defects. In clinical trials, 3% of patients developed an adverse response within 30 days to a 0.1-mL screening test dose (31). Of these, two thirds responded within 72 hours. This prompt response suggests a preexisting immunity presumably as a result of dietary exposure to beef products. Another 0.6% of patients who did not respond to the test subsequently reacted to a treatment dose. In a retrospective study that included 37 patients who responded locally to the test dose, 34 had circulating antibodies to bovine collagen that did not cross-react with human collagen (32). In a prospective study of 61 patients, two developed a treatment response and circulating antibodies to bovine collagen (33). In neither study was the development of antibodies related to any general systemic complaint.

The immune response is, of course, designed to remove or sequester foreign materials. In the few people immune to injectable collagen this seems to be exactly what is occurring. The collagen is rapidly cleared by the ensuing inflammatory process, and the worst effect beyond inflammation at the injection site would seem to be loss of material, if permanence were the desired result. Concerns are related to two potential problems. First, if the immune response is massive, secondary effects such as damage to organs, eg, kidney, by immune complexes can occur. This has not been observed when collagen is the antigen. Second, if a cross-reaction of antibodies to bovine collagen occurred with human collagen, an autoimmune disease might be precipitated. Although this is a theoretical concern, there is no evidence that it occurs with animal collagens in the form of sutures, hemostatic agents, and injectable collagen.

Antigenicity. The term antigenicity refers to the ability of a protein or other substance to react with an antibody. In general, proteins show two types of antigenic determinants, sequential and conformational. Sequential determinants depend only on the recognition of an amino acid sequence, typically 5–10 amino acids. Conformational determinants depend on the presence of a particular three-dimensional structure. Conformational antigenicity is lost when the protein is denatured, presumably because more than one region of the polypeptide chain(s) contributes to the antigenic site. In the case of type-I collagen, although the triple helix is a poor immunogen, some denaturation may take place *in vivo*, which

reveals sequential determinants. Indeed, it is common experience that polyclonal antibodies, either raised deliberately in animals or as a consequence of collagen utilization in humans, react with both native and denatured collagen. The telopeptide ends in soluble collagen are either unstructured or have differing structures. Thus they present sequential antigenic determinants that are frequently described as the principal antigenic determinants of collagen. However, this has been documented only in the rabbit, the most common experimental animal used for making antibodies. In other species, helical determinants may be equally important (30).

Isolation and Purification

Soluble Collagen. Type-I collagen is not normally extractable from tissues because it is cross-linked. However, in some tissues, notably the skin of young animals, cross-linking is sufficiently slow that a small portion, at most a few percent, can be extracted in cold 0.5–1 *M* NaCl at neutral pH. Somewhat more can be extracted by dilute acid, eg, 0.5 *M* acetic acid, which cleaves aldimine cross-links. Rat-tail tendon from young rats is unique with cross-linking being mostly of the aldimine type, and thus the collagen is almost completely extractable in dilute acid. The proportion of extractable collagen can be increased if the animal is fed β-aminopropionitrile, an inhibitor of peptidyl lysine oxidase, the enzyme that forms aldehydes on collagen. This compound induces the condition called lathyrism, referred to earlier. Although these procedures yield collagen sufficient for research studies, they are not adequate for large-scale purposes. Much higher yields of soluble collagen can be obtained by taking advantage of the fact that the collagen helix is relatively resistant to proteases below about 20°C. Proteases do, however, cleave peptide bonds in the telopeptide regions. Since cross-links originate in these same regions, molecules are freed from the matrix and become extractable. Pepsin is the favorite enzyme for this purpose because it is used at low ionic strength and acid pH, conditions that swell and solubilize collagen as peptide bonds are cleaved near cross-links. Soluble collagen prepared in this manner is slightly truncated, having lost the telopeptide ends, but under the right conditions the helix remains essentially intact. Procedures for preparing and characterizing soluble collagen have been reviewed (2,4).

Soluble collagen is purified mainly by precipitation. The high viscosity and nonideal behavior of even dilute solutions interfere with physical chemical methods such as chromatography, electrophoresis, and differential sedimentation. The solubility properties in aqueous solution that form the basis of precipitation methods are as follows (34): Type-I collagen monomer is soluble in the cold at pH <5 and salt concentrations below the equivalent of about 3% NaCl and at pH >5 and salt concentrations between the equivalents of 1–6% NaCl. Solubility near neutral pH is highly temperature dependent and shows an inverse relationship.

Collagen solutions contain varying proportions of monomer and higher molecular weight covalently linked aggregates, depending on the source and method of preparation (35). The solubilities of aggregates larger than a few molecules are sharply reduced under the conditions described above. Solubility forms the basis for separation on the basis of size, but truly monomeric solutions are difficult if not impossible to obtain. Pepsin-solubilized collagen usually contains a higher

proportion of monomer than salt- or acid-extracted collagens, but some covalent cross-links occur in regions not susceptible to proteases. Thus dimers, trimers, and higher aggregates are still present.

Once dried, soluble collagen may be difficult to resolubilize because, like other proteins, denaturation and nonspecific cross-linking occur readily. Lyophilized collagen can be redissolved at least for several months if properly stored (36).

Fibrillar Collagen. Extensively cross-linked collagen from, for example, skin or tendon can be dispersed as a fine fibrillar suspension by the use of mild denaturing agents, mechanical fragmentation, and selective proteolysis of noncollagenous components. The limited information available on these processes, which are often proprietary, has been recently reviewed (37,38). Such dispersions may even be clear to the eye, but the collagen is not in solution as can be ascertained if such preparations are filtered or centrifuged. Even so, dispersions can be at least partially purified by extraction methods and differential proteolysis. The fibrillar collagen is more insoluble and more resistant to proteolysis than most other tissue constituents. Reconstitution by salt or organic solvent precipitation, air drying, filtration, or lyophilization under appropriate conditions can yield powders, sheets, tubes, threads, mats, sponges, fleece, or molded forms. Stabilization can be achieved by cross-linking to the desired extent, usually with glutaraldehyde.

For sterilization, ionizing radiation is most commonly used (39), but other methods including dry heat and ethylene or propylene oxide are available. Because these methods are designed to destroy microorganisms, they are inherently damaging to proteins and alter the nature of the collagen. Materials prepared from reconstituted soluble collagen can be sterilized under mild conditions by filtration of the soluble collagen followed by aseptic processing and packaging.

Reconstituted Fibrillar Collagen. Collagen in solution can be reconstituted into fibrils that have a structure similar to that of native fibrils from which it was originally extracted (9). This self-assembly can be carried out by one of two procedures. If collagen dissolved in acid is converted to a low ionic strength solution near neutral pH, spontaneous precipitation occurs. For example, the collagen is dialyzed in dilute acetic acid at about 1 mg/mL against 0.02 *M* Na_2HPO_4 in the cold; precipitation is rapid and essentially complete. Pepsin-solubilized collagen as well as intact collagen forms fibrils by this procedure. Viewed by electron microscopy, the collagen fibrils usually have the characteristic band pattern of native fibrils. However, the narrow distribution of fibril diameters and the presence of fibril bundles characteristic of collagen *in situ* are not seen.

The second procedure involves the dissolution of collagen in the cold in a buffer close to physiological ionic strength and pH. This solution is stable for some time if the soluble collagen is relatively free of aggregates. However when warmed to 20–37°C, fibrils precipitate; the rate depends on collagen concentration, temperature, ionic strength, and molecules that inhibit or accelerate association. Because the rate can be regulated, this procedure has been widely used to study the mechanism of fibril formation, as illustrated in Figure 8. Fibrils formed by this procedure from intact soluble collagen may have the banded structure of fibrils *in situ*, but again, higher level tissue architecture is absent. However, pepsin-solubilized collagen forms nonbanded fibrils by this procedure

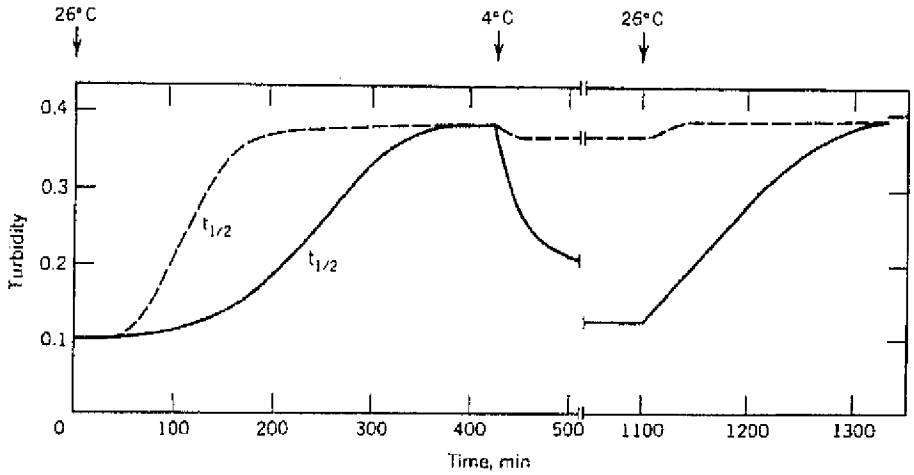


Fig. 8. Kinetics of collagen fibril formation *in vitro*. Rat-tail-tendon collagen in solution (0.1 mg/mL, pH 7.3, 0.225 ionic strength) at 4°C was warmed to 26°C at time 0 and cooled to 4°C when assembly was complete. Fibril formation was followed by turbidity (absorbence at 313 nm). --------, Normal collagen; ————, reduced collagen. Reduction converts the cross-link-precursor aldehydes to alcohols and prevents spontaneous cross-linking as assembly occurs. Cooling reverses assembly, but a second warming to 26°C initiates assembly without a lag phase (40). Courtesy of the *Journal of Biological Chemistry*.

(41). This phenomenon demonstrates the importance of the telopeptide ends in fibril structure. The fibrils usually form a homogeneous gel, allowing assembly to be followed by turbidity. The turbidity curves (Fig. 8), which have lag and growth phases, eventually reach a plateau. Collagen that contains preformed aldehydes spontaneously cross-links, and assembly is irreversible. If aldehydes are absent or reduced, cooling reverses assembly. The kinetics can be treated formally by nucleation-growth theory (42), but the actual mechanism of self-assembly is undoubtedly more complex (43).

Collagenous Tissues. Tissues consisting largely of collagen, including skin, tendon, dura, large veins, and heart valves, can be cleaved mechanically by extraction and sometimes by selective proteolysis (38). These tissues already have a fixed form, and the collagen retains its original tissue architecture. Thus for medical applications, the normal biological form and, one hopes, function is available. Human tissue is, of course, preferred for use in humans, as it reduces the potential for immunogenicity but is usually not available in sufficient quantity. Animal tissue may be used in some instances, particularly after proteolysis to remove noncollagenous proteins or extensive glutaraldehyde cross-linking to reduce solubility and thus immunogenicity.

Characterization

Denatured Collagen

When collagen in solution is heated above its melting point, it denatures or melts. The chains dissociate and remain in solution if the temperature is kept

above the melting point. This temperature is commonly about 40°C for mammalian collagens but may be lower if denaturing agents such as urea, guanidine·HCl, or $CaCl_2$ are used. Characterization of the components derived from soluble collagen by controlled denaturation provides useful information about the native protein because the covalent structure is retained. Furthermore, these components are soluble and behave ideally in transport procedures, as described below, unlike native collagen. Only the most useful characterization methods are considered here. More detailed accounts and other methods are described elsewhere (2,4,34).

Molecular-sieve Chromatography. This procedure separates on the basis of molecular weight. Because the uncross-linked collagen molecule contains three α chains of the same size, a molecular-sieve chromatogram of this idealized protein would show a single peak. In practice all soluble collagen preparations contain varying degrees of cross-linked α chains, which may arise from both intra- and intermolecular cross-links. For historical reasons α-chain dimers are referred to as β-components, and trimers as γ-components. These components as well as larger aggregates can be observed in the chromatogram reproduced in Figure 9.

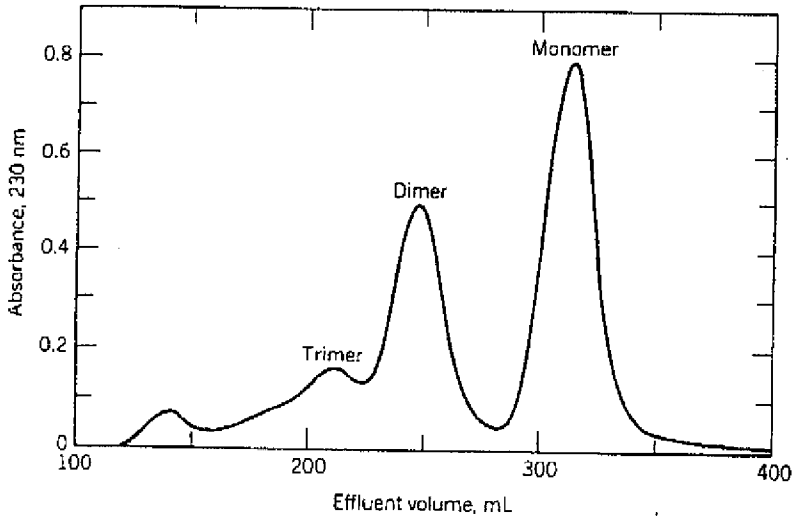


Fig. 9. Separation of the components in denatured rat-tail-tendon collagen by molecular-sieve chromatography. Monomer is single α chains, M_r = 95,000; dimers, trimers, and higher aggregates arise from covalent cross-links (35). Courtesy of the *Journal of Biological Chemistry*.

Recently, molecular-sieve material has been adapted to high pressure liquid chromatography technology, where it increases speed and sensitivity (44). The main advantage of molecular-sieve chromatography is that it provides an accurate measure of the distribution of covalently cross-linked components in a sample of denatured soluble collagen (see also CHROMATOGRAPHY).

Carboxymethyl Cellulose Chromatography. Denatured collagen components are slightly basic, and as such, chromatograph well on a carboxymethyl cellulose cationic exchanger at about 40°C. Because separation is based largely

on charge density, the two types of α chains in type-I collagen, $\alpha 1$(I) and $\alpha 2$(I), can be separated as shown in Figure 10. Their 2:1 ratio is readily apparent. Unfortunately, because cross-linked components have charge densities similar to α chains, overlap occurs. The method is suitable only for soluble collagens that are not extensively cross-linked or after fractionation by molecular-sieve chromatography.

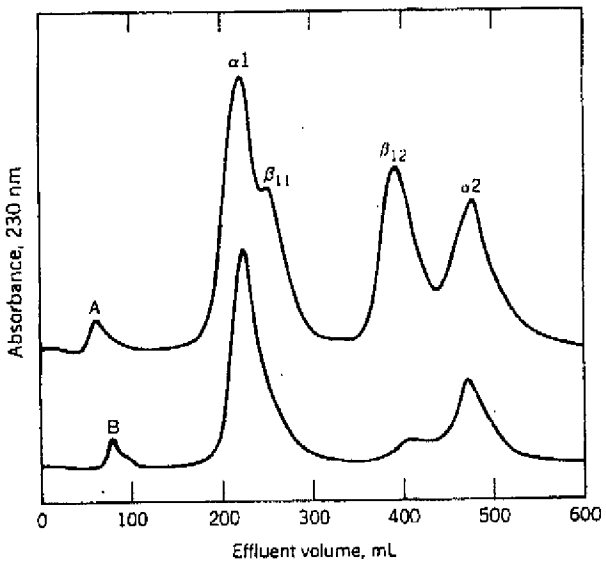


Fig. 10. Carboxymethyl cellulose chromatogram of denatured soluble pig-skin collagen. A, acid-extracted collagen; B, salt-extracted collagen; $\alpha 1$ and $\alpha 2$ are single chains, M_r = 95,000; β_{11} is a cross-linked dimer of two $\alpha 1$ chains; and β_{12} is a dimer of one $\alpha 1$ and one $\alpha 2$ chain. The salt-extracted collagen contains fewer cross-linked components (45). Courtesy of Butterworth Publishers, Inc.

Sodium Dodecylsulfate (SDS) Gel Electrophoresis. This method provides separation on the basis of molecular weight on the assumption that proteins bind equivalent amounts of SDS. However, collagen chains bind less SDS than most proteins (46) and thus appear to have higher molecular weights than actual when compared to globular proteins. Furthermore, the $\alpha 1$(I) and $\alpha 2$(I) chains bind SDS to different degrees and thus separate even though they have the same molecular weight. The result is fortuitously high resolution, as shown in Figure 11. For this reason, and to provide sensitivity and adaptability to multiple analyses, SDS gel electrophoresis has largely supplanted other separation procedures not only for denatured collagen but for all proteins. However, the method is difficult to quantitate, recoveries cannot be easily determined, and the high-contrast dye-staining procedures usually employed often fail to reveal minor components. The last difficulty has been largely overcome by the introduction of a sensitive silver-staining procedure (47), but quantitation and the calculation of recoveries remain problems. The method is best suited to monitor other processes rather than as a primary separation procedure (see also ELECTROPHORESIS).

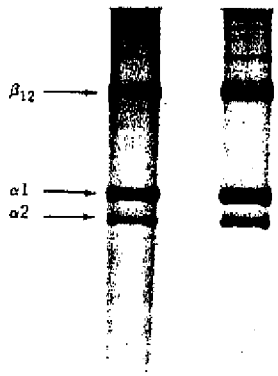


Fig. 11. Sodium dodecylsulfate polyacrylamide gel electrophoresis of denatured pepsin-extracted bovine-skin collagen (a) before and (b) after purification by precipitation in 0.02 *M* Na_2HPO_4. The components are labeled as in Figure 10. The silver stain (47) brings out degradation products, (a) diffuse background, produced by pepsin digestion that are largely removed by precipitation (b). Electrophoretogram courtesy of John M. McPherson, Collagen Corporation.

Soluble Collagen

Native type-I collagen in solution, usually at acid pH, has been extensively characterized by physical techniques including viscosity determination, ultracentrifugation, sedimentation equilibrium, electrophoresis, intensity light scattering, dynamic light scattering, electric birefringence, nuclear magnetic resonance, optical rotary dispersion, circular dichroism, and electron microscopy of rotary-shadowed molecules (see also CHARACTERIZATION OF POLYMERS). The results of these studies have been reviewed extensively (48,49). Because this literature is readily accessible, the discussion here is limited to viscosity, denaturation as measured by optical rotation, and electron microscopy of rotary-shadowed molecules. These were chosen because they are particularly informative when applied to collagen in solution and the techniques are not difficult to apply.

The collagen molecule is rodlike but has considerably more flexibility than might be expected. Its molecular weight is 290,000 and its dimensions are 300 × 1.5 nm. Solutions always contain some preexisting covalently linked aggregate, often in large amounts, which may dominate the parameter being measured. The molecule is triple helical and easily denatured in a manner that is irreversible under most conditions. At acid pH and moderate ionic strength,

molecules do not aggregate, but at near neutral pH, aggregation predominates with an inverse temperature and inverse ionic strength dependence.

Viscosity. Because of the very high asymmetry of the collagen molecule, the intrinsic viscosity of collagen monomer in acidic solution is large, approximately 10^3 mL/g. This value is sensitive to the presence of denatured collagen, which has an intrinsic viscosity on the order of 50 mL/g, and to the presence of aggregates. The effects of aggregates are complicated because to a first approximation, aggregates of lower asymmetry reduce the viscosity, whereas aggregates of higher asymmetry increase the viscosity. The observed effect is that the presence of aggregates increases the intrinsic viscosity but has a greater effect on the concentration dependence of the reduced viscosity (see Fig. 12).

The measurement of viscosity of acidic collagen solutions is relatively easy because of the large values observed. Low collagen concentrations can be used, making determination of the concentration dependence unnecessary. However,

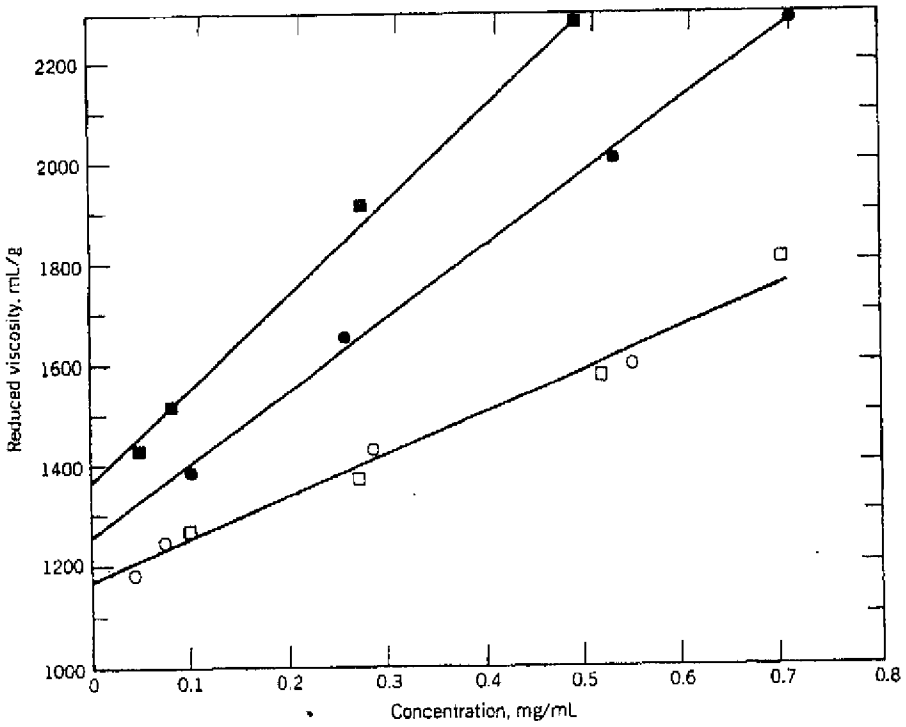


Fig. 12. Reduced viscosity of rat-skin collagen solutions as a function of concentration. The collagen was either salt-extracted (○,●) or acid-extracted (□,■) before (■,●) or after (□,○) limited chymotrypsin digestion. Solvent was 0.15 *M* potassium acetate, pH 4.8. The higher intrinsic (extrapolated) viscosity and concentration dependence of acid-extracted collagen result from an increased proportion of higher aggregates obtained by the more vigorous extraction method. Aggregates were proteolytically dissociated by chymotrypsin cleavage in the telopeptide regions, reducing the viscosity and the concentration dependence (50). Courtesy of *Biochemistry*.

the information given by the concentration dependence may be useful. Capillary viscometers with moderate shear gradients can be used for comparative studies, but more accurate values are given by rotating-cylinder viscometers because they have inherently low shear gradients (48).

Denaturation as Measured by Optical Rotation. Collagen in solution has a characteristic optical rotary dispersion as a result of its triple helix. The very large negative rotation of native collagen relative to denatured collagen makes optical rotation at a fixed wavelength a sensitive measure of denaturation (49). Wavelengths in the ultraviolet provide very high sensitivity, but for most purposes wavelengths in the visible region are satisfactory. Thus it is relatively easy to measure the helical content of collagen in solution and to study the nature of the helix by the melting transition obtained by increasing the temperature. The instrumentation needed is a polarimeter and recorder set to record rotation over time, a water-jacketed polarimeter cell, and a programmable circulating water bath; typical melting curves are shown in Figure 13.

Curves such as these are analyzed as follows: First, the specific rotation (ca $-125°$ at 489 nm and 45°C) of denatured type-I collagen (mammalian) can be determined if the concentration is known. The melting curve is normalized to this value, giving specific rotation values at all temperatures. The optical rotation at temperatures where no melting has occurred is a measure of helical content. The expected value (ca $-400°$ at 489 nm and 25°C) can be determined on known intact collagen. The nature of the helix is indicated by the midpoint of the transition T_m and the width of the transition, measured as the temperature between one-quarter and three-quarters denatured; T_m varies slightly with the solvent and the source of the collagen but is near 40°C for intact mammalian type-I collagen. The width of the transition depends somewhat on the heating rate but is commonly 1–2°C. Any deviation from these values, compared to internal standards, indicates the presence of denatured collagen or of altered collagen melting at a lower temperature. If the lower temperature is sufficiently different from T_m, the melting transition appears multiphasic. Such a situation is more easily analyzed from the first derivative plot of the melting transition (Fig. 13b).

Electron Microscopy of Rotary-shadowed Collagen Molecules. In this technique (51) a dilute solution of a protein in a saltfree or volatile salt solution is sprayed as a fine mist onto cleaved mica. The mica is placed in a vacuum chamber and a heavy metal such as platinum is evaporated onto the mica at a low angle while the mica rotates. Metal piles up against protein molecules lying on the mica. The size and shape of the protein molecule can be determined from the metal outline by transmission electron microscopy. The technique has recently been markedly improved (hence its resurgence), by adding about 50% glycerol to the protein solution (52). Water, of course, evaporates rapidly in the vacuum chamber but glycerol does not. Apparently, as a result, the protein molecules do not collapse as readily as they do in water alone, and their higher profiles produce denser outlines and sharper contrast.

Electron micrographs of rotary-shadowed pepsin-solubilized molecules of bovine-skin collagen are shown in Figure 14; also shown is a characteristic covalently linked dimer. By measuring molecules in enough fields to give good statistical values, a soluble collagen can be characterized with regard to content and nature of aggregates; shortened or truncated molecules can also be detected.

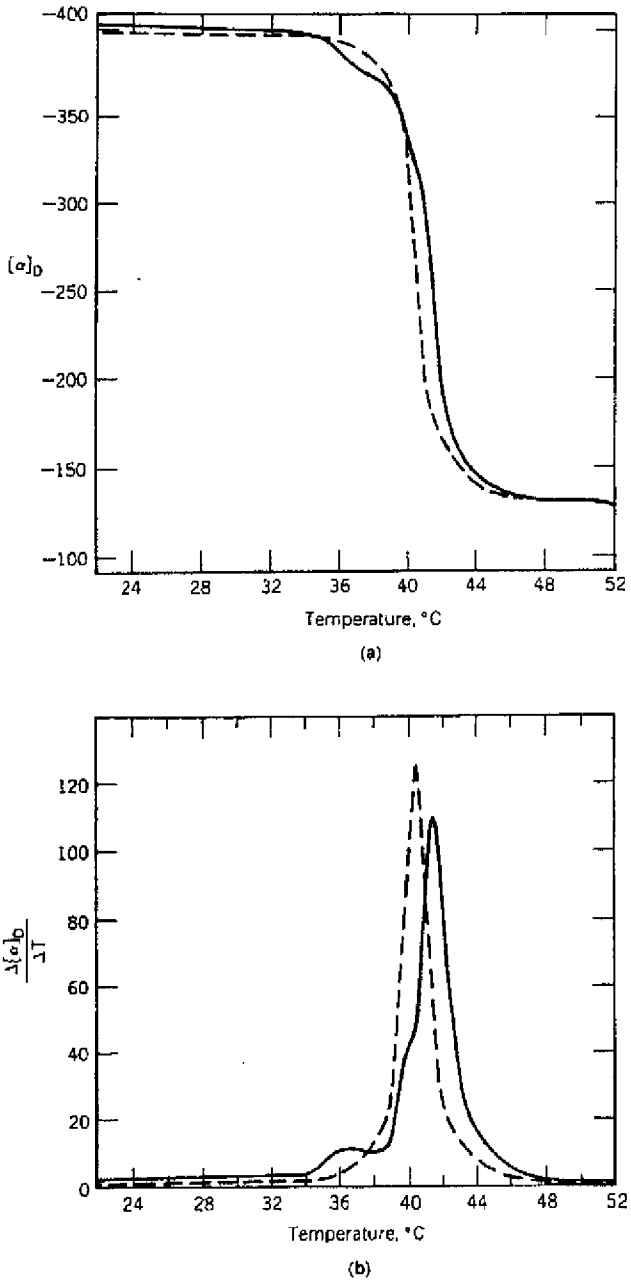


Fig. 13. (a) Melting curves of rat-tail-tendon collagen (-----) and of pepsin-extracted bovine-skin collagen (————) as determined by optical rotation. (b) First derivative plots of the melting curves showing the presence of low melting components in the pepsin-extracted collagen. The difference in melting temperatures (ca 1°C) of the main components is probably real and may arise from a tissue or species difference. The collagen concentrations were 2.3 mg/mL (bovine skin) and 2.8 mg/mL (rat-tail tendon) in water at pH 2 (HCl). The solutions were heated at a linear rate of 0.4°C/min in a 10-cm polarimeter cell. Data courtesy of Donald G. Wallace, Collagen Corporation.

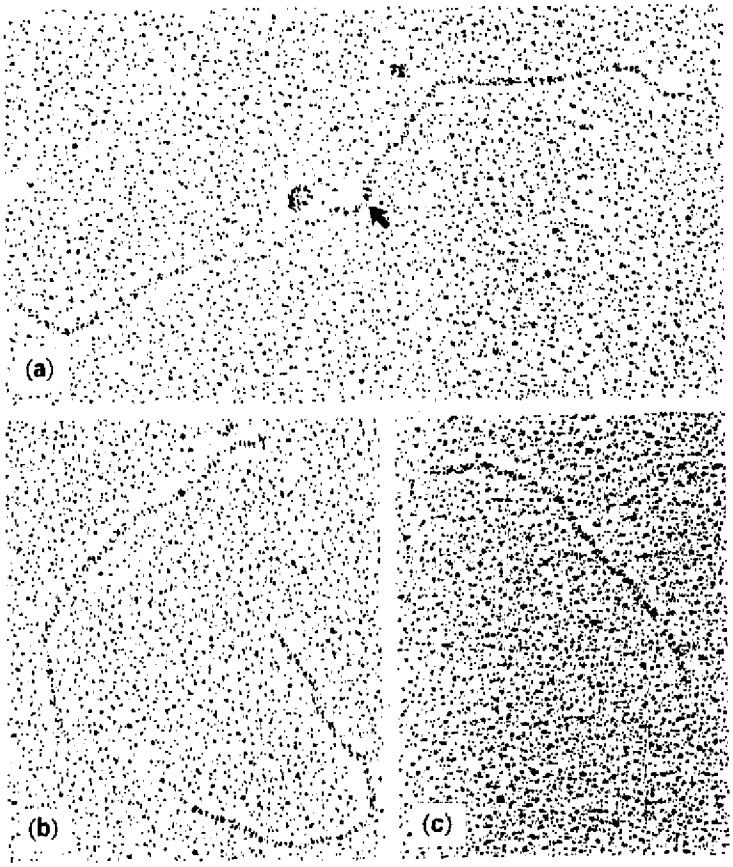


Fig. 14. Electron micrographs of rotary-shadowed collagen molecules obtained by pepsin digestion of calf skin. (**a**) An end-linked (arrow) dimer about 600 nm long; (**b**) and (c) single molecules about 300 nm long. Shadowing was with platinum–carbon at an angle of 7°. Electron micrographs courtesy Joseph A. Madri, Yale University.

Fibrillar Collagen

Insoluble collagen in fibrillar form, whether never solubilized or solubilized and reconstituted, is more difficult to characterize since most physical methods require protein in solution (53,54). In addition, the form of the collagen as well as its inherent properties are usually of interest. For example, it is usually important to measure macroscopic parameters such as density, water content, and solubility. Other parameters such a surface area, water uptake, tensile strength, compressive strength, rheological properties, and scanning electron microscopic appearance may be useful. These tests must be specialized to the form of the material and the particular solid-state physical properties required by the application.

The most revealing characterization method for insoluble collagen is x-ray diffraction (8–10). However, oriented collagen fibrils are usually necessary but

often not obtainable. In addition, x-ray diffraction is a research tool not adaptable to routine characterization tests. However, three useful characterization methods are readily adapted to all fibrillar (or otherwise insoluble) collagens. These are amino acid analysis, differential scanning calorimetry, and transmission electron microscopy.

Amino Acid Analysis. The idealized compositions of type-I collagen α chains are given in Table 1. In an actual analysis, these values are normalized to some total number of residues, usually 1000. It is characteristic that collagen contains much more glycine (exactly one third), proline, and alanine than most proteins, in addition to hydroxyproline and hydroxylysine, which are absent from most other proteins (2,55). Thus the presence of other proteins can be ascertained by amino acid analysis. Unfortunately, because the precision of any analytical value is at best a few percent in routine assays, small amounts of other proteins are not detectable. The tyrosine value is often significant (35) because tyrosine is present in smaller amounts than in most proteins, being found essentially only in the telopeptide ends of collagen. Higher than normal tyrosine values indicate the presence of other proteins; lower than normal values reflect loss of the telopeptide ends.

Differential Scanning Calorimetry. This procedure measures the heat absorbed (or released) as the temperature of a sample is raised at a constant rate (56). Since melting of collagen is endothermic, a negative peak or peaks are obtained corresponding to the denaturation temperature(s) of the component(s)

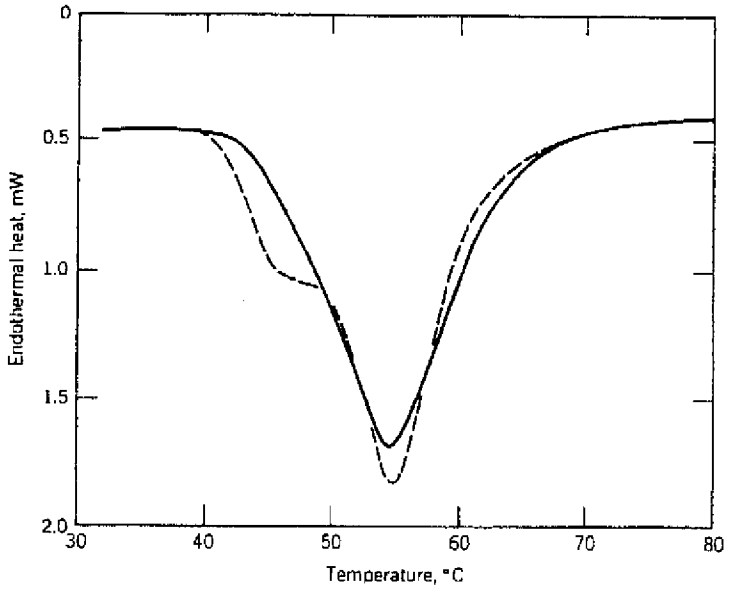


Fig. 15. Differential scanning calorimetry of reconstituted fibrillar collagen from acid-extracted rat-tail-tendon collagen (————) and pepsin solubilized calf-skin collagen (------). Fibrils were reconstituted in 0.02 *M* phosphate by neutralizing an acid solution at 20°C and adding NaCl to 0.13 *M*. The pellets obtained by centrifugation were heated at 10°C/min in a Mettler TA3000 calorimeter with a DSC 20 cell. The inhomogeneity of fibrils formed from pepsin solubilized collagen is evident. Data courtesy of Richard Condell, Collagen Corporation.

present. For intact collagen in solution, the curve is essentially the same as the first derivative plot of the melting of collagen, as observed by optical rotation (Fig. 13). As shown in Figure 15, insoluble collagen may present a more complex picture, since denaturation involves more than triple-helix melting. Specifically, a fibrillar collagen is stabilized by interactions between molecules as well as within the triple helix. Thus if the insoluble collagen is homogeneous, it melts at a higher temperature than in solution (about 55°C depending on conditions) but still shows a single transition. In actual fact, most insoluble collagens present a complex picture because of structural inhomogeneity. Inhomogeneities may arise at the molecular level or at the fibril level, as a result of differences in packing. The largest changes may be caused by extremes of processing such as, for example, heat or irradiation used for sterilization. Thus differential scanning calorimetry may have to be used empirically, but it is very useful in comparing the integrity of an insoluble collagen to a known standard.

Electron Microscopy of Thin Sections. Collagen fibrils have characteristic bands. Suspension preparations of fibrils (Fig. 3) provide the highest resolution and the most information about individual fibrils (9,10). However, the process of fibril dispersion and attachment to a grid prior to microscopy of this kind selects information for certain types of fibrils (dissociable and not too narrow or wide) and destroys information about fibril relationship. Furthermore, dispersion at this level is not always possible. Electron microscopy of thin sections is a much more general procedure, avoids the selection problem as long as enough regions and sections are viewed, and provides information about fibril relationship as well as individual morphology. The technique is reasonably standard and reproducible. The steps include cutting a tissue block or if the sample is a suspension, centrifuging to form a pellet; fixing; embedding in plastic; sectioning at about 50 μm; staining; and viewing in the electron microscope. Examples of a reconstituted fibrillar collagen suspension and of normal skin are shown in Figure 16.

The information that can be obtained from electron micrographs such as these is usually limited to qualitative impressions. Although only large differences can be readily documented, they can occur and may include complete loss of fibril-band pattern or even fibril identity under extremes of processing. Thus electron microscopy is most useful in understanding the effect of processing on collagen structure. By using standard morphometric methods, it is also possible to measure fibril diameter and area occupied by fibrils.

Uses

The technology used to manufacture articles of commerce such as sutures, hemostatic agents, tissue replacements, and sausage casings from fibrillar collagen is as much art as science. It is not reported in the literature but is the subject of many patents. Most process information is proprietary.

Industrial uses of collagen (56) as gelatin and leather are undoubtedly the largest. Another industrial use not generally realized is sausage casings made of dispersed insoluble collagen extruded as a tube and formed or cut into links as it is filled. The patent literature is the best source of information on this subject. Collagen is also an ingredient in some cosmetics. However, the amounts present are small and the collagen is degraded and probably more appropriately classed as a gelatin. Although collagen may add some useful physical qualities

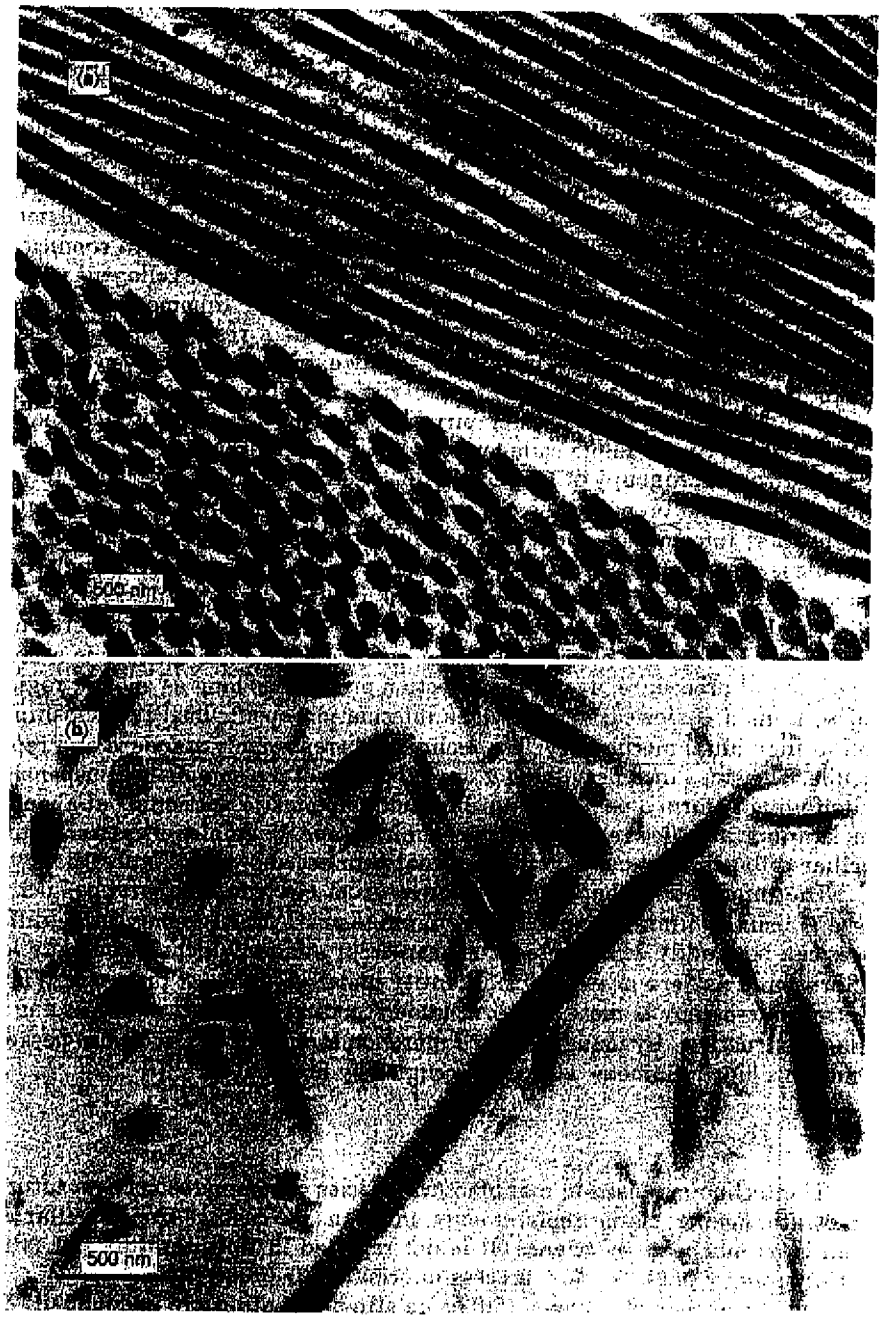


Fig. 16. (a) Electron micrograph of normal human skin showing homogeneity of collagen fibril diameter and fibril bundle organization. (b) Electron micrograph of fibrillar collagen reconstituted from pepsin-extracted bovine-skin collagen and incubated at 37°C for 24 h in 0.13 *M* NaCl, 0.02 *M* phosphate, pH 7.4. The fibrillar suspension was 35 mg/mL. Both samples were fixed in glutaraldehyde, embedded in plastic, sectioned at about 50 nm, and stained with uranyl acetate and lead citrate. Electron micrographs courtesy of Shirley Chu, Collagen Corporation and Klaus Bensch, Stanford University.

to the cosmetic, they are unrelated to the biological properties of collagen and the function they serve can only be external to the body.

The most interesting applications of collagen are probably biomedical (37,38,57–61) (see Table 2). The attractiveness of collagen as a biomaterial rests largely on the view that it is a natural material of low immunogenicity and is therefore seen by the body as a normal constituent rather than foreign matter. Although this view is reasonable and provides a rational starting point, the processing that collagen must undergo for conversion to useful forms unavoidably changes its properties and may lessen its apparent advantage over other polymers. To process collagen and use it effectively it is necessary to understand the starting material.

Table 2. Biomedical Applications of Collagen

Application	Collagen form
hemostatic agents	powder, sponge, fleece
blood vessels	processed human or animal blood vessel, reconstituted fibrillar collagen
heart valves	processed porcine heart valve
tendons and ligaments	processed animal tendon, collagen–carbon fiber composite
burn dressings	processed animal skin, sponge, composites
intradermal augmentation	reconstituted fibrillar collagen
drug-delivery systems	various biodegradable forms

Acknowledgment

The author is indebted to Susan Kweskin for help in assembling information used in this chapter and for editing of the manuscript. Thanks are also due to the many colleagues who have provided information and advice.

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